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SPOTLIGHT

The long and short of membrane curvature sensing by septins

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Septin proteins form hetero-oligomers that associate with membranes of specific curvatures, but the mechanism is unknown. In this issue, Cannon et al. (2019. *J. Cell Biol.* https://doi.org/10.1083/jcb.201807211) identify a single amphipathic helix that is necessary and sufficient for membrane curvature sensing by septins.

Cells and most organelles inside them are bounded by membranes typically composed of lipid bilayers. In phospholipids, the most common lipids in biological membranes, the phosphate "heads" prefer aqueous environments, and the "tails"—two fatty acid chains—pack together with other such tails, creating bilayers with the tails on the inside and heads packed together on the outer surfaces (Fig. 1 A). Pure lipid membranes eventually form spheres in aqueous solutions. Cells and organelles, on the other hand, are rarely spherical, due in part to local changes in membrane curvature resulting from distinct lipid compositions in the two layers and/or to the influence of membrane-associated proteins.

An amphipathic helix (AH), which has one hydrophilic and one hydrophobic face, brings a protein to a membrane by "submerging" the hydrophobic face in the membrane, with the helix oriented parallel to the membrane surface like a spoon skimming the fat from soup. Like the spoon in the soup, the hydrophobic amino acid sidechains of the AH disturb the surface of the membrane. In a precurved membrane the lipids are packed together differently in one layer than the other, and these lipid-packing defects are thought to make it easier for the hydrophobic AH sidechains to insert (2). Thus an AH can allow a protein to "sense" membrane curvature.

In many cases, these curves are rather "tight": imagine a vesicle budding from the plasma membrane into the cytosol during endocytosis. But others are comparatively shallow, such as the ∼1-µm-diameter neck where a *Saccharomyces cerevisiae* mother cell sprouts a bud (Fig. 1 B). At the yeast bud neck curvature actually inverts from "negative" to "positive" (imagine going from concave to convex). How do proteins recognize membranes with such micron-scale curvatures?

Through studies pioneered by the laboratory of Amy Gladfelter (3), septin proteins have recently emerged as sensors of micron-scale membrane curvature in eukaryotes. Individual septin proteins coassemble into rod-shaped hetero-oligomers. Most septins have a helix near the N terminus with positively charged sidechains clustered on one face, and within a hetero-oligomeric septin rod these "polybasic" motifs decorate one side of the rod (Fig. 1 A) where they can stick to negatively charged phospholipid head groups. Once stuck loosely to a membrane surface, septin rods bump into each other and "anneal" end-to-end to form filaments (4).

An array of septin filaments forms at the *S. cerevisiae* bud neck (5), where septins were first discovered, but why septin filaments do not form elsewhere in these cells was unclear. The Gladfelter laboratory previously noticed that in a related fungus, *Ashbya gossypii*, septins localize to membrane regions of a narrow range of positive curvatures where filamentous *Ashbya* cells make "branches" (3). They further found that purified yeast septins bind to lipid bilayer–coated beads of sizes that match the curvature preferences seen in vivo (4). So how do septins sense curvature? Unpolymerized (i.e., nonfilamentous) septin rods bind beads poorly but still exhibit a curvature preference, indicating a curvature-sensing mechanism intrinsic to the rod, but the molecular details were unresolved.

In this issue, Cannon et al. (1) first confirmed that stable septin-rod-to-membrane association is cooperative in vitro, and filaments bind tighter than do rods. Switching from fluorescence microscopy with spherical beads to scanning EM with bilayer-coated cylinders allowed the authors to directly observe that when filaments were longer and packed together side-by-side, they preferred a narrower range of curvatures. Though the orientation of short filament alignment was sometimes different by 90° from that of long, laterally associated bundles, even very short filaments (approximately two rods long) clearly aligned themselves to bind micron-scale positive curvature. Phospholipid composition had little effect on curvature preference, and when the authors directly watched single fluorescent rods arrive and depart from a curved surface, it was clear that the curvature preference reflected faster association. Yet the mystery remained: How do individual rods sense curvature?

A search for predicted septin AHs found several, including an evolutionarily conserved one at the C terminus of the yeast septin Cdc12 (Fig. 1 D). Remarkably, a mutant truncating this sequence was isolated decades ago in an unbiased screen and represents a commonly used genetic tool, since in the mutant cells higher-order septin assemblies disappear rapidly upon a shift to high temperature, but the molecular basis was unknown. The Cdc12 AH was found to be both necessary and sufficient for

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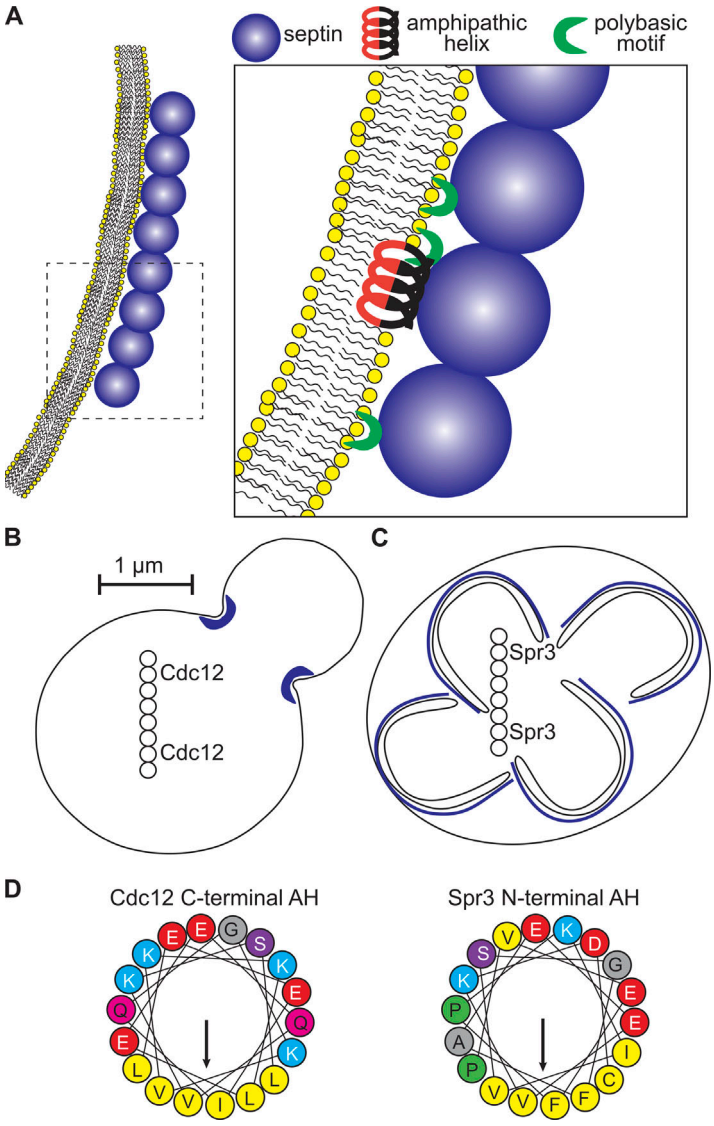

Figure 1. **Septin complexes interact with curved membranes via an AH. (A)** Cartoon of a single septin hetero-octamer interacting with a curved membrane bilayer via polybasic motifs and an AH. Not drawn to scale. **(B and C)** Budding yeast septin complexes (blue) have distinct subunit composition and interact with membranes of distinct curvatures during budding (B) or sporulation (C). **(D)** Helical wheel representations of the C-terminal AH in Cdc12 shown by Cannon et al. (1) to mediate curvature-sensitive membrane interaction, compared with an N-terminal AH in the sporulation-specific counterpart Spr3. Arrows show the direction and relative magnitude of the hydrophobic dipole moment, an indicator of overall amphipathicity.

curvature preference in vitro, and when the authors made an equivalent truncation mutation in the *Ashbya* homologue of Cdc12, septin association with curved membranes in vivo was drastically reduced (1).

Another recent study (6) independently discovered in human Sept9 two predicted AHs, one adjacent to (partially overlapping) the known polybasic motif, and another next to a previously uncharacterized polybasic motif. The polybasic motifs turned out to be important for septin polymerization, and it was the AHs that mediated membrane association. Yet another recent study (7) exploited synthetic vesicles and "wavy" membrane surfaces to come to essentially the same conclusions as Cannon et al. (1) in terms of yeast septin filament length/orientation/packing and curvature preference. The new vesicle experiments provide evidence supporting much older observations (8) that septin binding can induce membrane curvature, hinting that septins may be not just sensors but also sculptors of curved membranes. Finally, Beber et al. derive a mathematical model that may reconcile a long-standing controversy in the yeast septin field regarding how to explain observed 90° "rotations" in septin filament orientation at the yeast bud neck: as membrane curvature changes during cytokinesis, septin filaments should realign correspondingly (7).

During sporulation, a form of gametogenesis, yeast septins associate with membranes of distinctly different curvature, and the findings of Cannon et al. (1) provide a compelling rationale for why Cdc12 is mostly evicted from septin rods and replaced by a sporulation-specific septin, Spr3 (9; Fig. 1 C). Spr3 may convey on septin rods a distinct curvature preference appropriate for this developmental stage. Indeed, Spr3 lacks a predicted AH at the C-terminal location equivalent to that of Cdc12, though it should be noted that in Spr3's extended N terminus a predicted AH can be found (Fig. 1 D) and represents an interesting target for future study.

AHs do not generally require specific phospholipids for membrane association, and septin curvature preference in vitro is at least partially independent of phospholipid identity. However, a recent study (10) shows that when human septins bind to the curved ends of rod-shaped bacterial cells in the context of bacterial infection,

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they are recruited there by cardiolipin, a curvature-specific bacterial membrane lipid. One can thus imagine how populating septin hetero-oligomers with subunits that direct interactions with membranes of specific curvatures represents a broader strategy for producing distinct complexes appropriate for a diverse set of cellular membranes. Given the diversity of septin subunits encoded in the human genome (13 genes, most encoding multiple protein isoforms), there is clearly much interesting work left to be done in this burgeoning field.

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